

Toxicity of cadmium on the germination of thyme seeds (*Thymus vulgaris* L.)

Cádmio tóxico na germinação de sementes de tomilho (*Thymus vulgaris* L.)

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Abstract

The accumulation of toxic metals (e.g., cadmium) in soil and water, and consequently in the food chain, is a concern in agriculture production due to the adverse effects of these metals on food quality and crop growth, as well as the potential harm they pose to humans and other animals. The objective of this work was to assess the toxicity of cadmium (Cd) on seed germination and initial growth of thyme. The ecotoxicological effects of four Cd concentrations (15; 30; 45; and 60 mg L⁻¹) were evaluated. The response variables were germination percentage, first count, germination speed index, total length, shoot length, root length, seedling dry mass, and tolerance index. The study found that thyme seeds exposed to 60 mg L⁻¹ of cadmium had a lower percentage of normal plants, a 13% reduction in seed germination, and a 65% increase in abnormal plants. The Cd had an inhibitory effect on the initial growth of the seedlings by affecting the development of the roots and aerial part. It is concluded that the presence and accumulation of Cd in the cultivation substrate reduced seed germination and initial seedling growth of thyme, and that the intensity of inhibition was directly proportional to the concentration of Cd in the solutions employed.

Resumo

A acumulação de metais tóxicos, como o cádmio, nos solos na água e, consequentemente, na cadeia alimentar, é preocupante na produção agrícola devido aos seus efeitos adversos na qualidade dos alimentos e no crescimento das culturas com um potencial significativo para prejudicar os animais e a saúde humana. Assim, objetivou-se com este trabalho avaliar a toxicidade do cádmio (Cd) na germinação de sementes e no crescimento inicial de tomilho. Foram avaliados os efeitos ecotoxicológicos de quatro concentrações de cádmio (15; 30; 45 e 60 mg L⁻¹). As variáveis respostas foram percentual de germinação, primeira contagem, índice de velocidade de germinação, comprimento total, da parte aérea e da raiz, massa seca das plântulas e índice de tolerância. Os resultados reportaram que as sementes de tomilho expostas ao cádmio apresentaram decréscimo do seu percentual de plântulas normais na concentração de 60 mg L⁻¹, com redução de 13% do percentual germinativo e aumento de plântulas anormais (65%). O efeito inibitório do Cd no crescimento inicial das plântulas afetou o desenvolvimento das raízes e da parte aérea. Conclui-se, portanto, que a presença e acumulação do Cd no substrato de cultivo reduziu a germinação de sementes e o crescimento inicial das plântulas de tomilho, sendo a intensidade da inibição diretamente proporcional à concentração das soluções empregadas.

Keywords

Cadmium chloride.
Germination process. Toxicity.

Palavras-chave

Cloreto de cádmio. Processo germinativo. Toxicidade.

1. Introduction

The contamination of soil and water with toxic metals is a serious environmental problem with consequences on the health of humans and other animals. Among these metals, cadmium (Cd) is a highly toxic element that is released into the environment through agricultural practices (e.g., the application of pesticides and chemical fertilizers, and irrigation with wastewater), burning coal, residues from smelting and mining, producing and improperly discarding batteries (e.g., from calculators and cell phones), and many other industrial processes (Amirjani, 2012; Alloway, 2013; Gill et al., 2011; Nikolić et al., 2014).

Humans can take in Cd by inhalation in an industrial environment or by ingesting contaminated food. After absorption, Cd is distributed by blood cells and can accumulate for a long time, mainly in the kidneys and liver, where its half-life can vary from around 10 to 40 years (Fernandes & Mainier, 2014). In addition, Cd is easily absorbed by roots and transported to the aerial part of plants, entering the food chain where it can be a serious threat to human health (Gill et al., 2011). Exposing plants to toxic quantities of Cd effects germination, compromises growth and development, interferes with various physiological processes, and decreases agricultural productivity (Guimarães et al., 2008).

Cadmium naturally occurs in all soils as a divalent cation (Cd^{2+}) that varies in concentration from 0.1 to 1.0 mg kg^{-1} (Smolders & Mertens, 2013). In plants, its concentration normally varies from 0.03 to 0.1 mg kg^{-1} and it is estimated that phytotoxic concentrations vary from 5 to 10 mg kg^{-1} in sensitive species (Kabata-Pendias, 2010). This metal can accumulate in all parts of a plant, which can result in the following: inhibition of seed germination; reduction of the root system and aerial part; disturbance of metabolic activities; interference with cell membrane permeability, decreasing the respiration rate; inhibition of photosynthesis; chlorosis; reduction in cell division and plant biomass; lipid peroxidation; and inhibition of enzymatic activities, harming the absorption and transport of water and essential elements inside the plant (Kabata-Pendias, 2010; Rahoui et al., 2010; Gill et al., 2013; Chen et al., 2014).

Some plant species can exhibit symptoms of toxicity when exposed to toxic metals, whereas other species can survive and adapt to the stress caused when these elements are in the soil, maintaining growth and development without exhibiting symptoms (Ovenčka & Takáč, 2014). Investigations of plant development under different levels of Cd contamination have been conducted, for example, with *Salix humboldtiana* (Gomes et al., 2011); *Helianthus annuus* (Amirjani, 2012); chamomile - *Matricaria chamomilla* (Saderi & Zarinkamar, 2012); wheat - *Triticum aestivum* (Ahmad et al., 2012); bean - *Phaseolus vulgaris* (Santos et al., 2013); *Solanum nigrum* (Chen et al., 2014); barley - *Hordeum vulgare* (Kalai et al., 2014); wheat and corn - *Zea mays* (Nikolić et al., 2014); *Diathus barbatus* e *D. chinensis* (Grigoraş & Stratu, 2015); *Brachiaria brizantha* e *B. decumbens* (Borges et al., 2016); wheat and bean (El Rasafi et al., 2016); chickpeas - *Cicer arietinum* (Ahmad et al., 2016); sesame - *Sesamum indicum* (Pires et al., 2016); basil - *Ocimum basilicum* - (Gharebaghi et al., 2017); *Schinus terebinthifolius* (Silva et al., 2017), among others. However, no studies of the toxic effects of Cd on seed germination of thyme were encountered.

Thyme (*Thymus vulgaris* - Lamiaceae) is cultivated from seed, native to the western Mediterranean of Europe and cultivated in southern and southeastern Brazil, where it is highly important medicinally and as a spice. It is a perennial subshrub, aromatic and 20 to 30 cm tall, with branches lightly covered by white hairs and simple leaves (Nascimento et al., 2000). The plant has a strong fragrance and has excellent bronchopulmonary and antiseptic properties, making it highly effective at treating respiratory problems. Its principal components are phenols, thymol (40%), and carvacrol (15%) (Segvić et al., 2007). Carvacrol has been studied for its bactericidal effect, and thymol is notable for its antifungal and antibacterial effects (Vieira de Melo et al., 2000). Under cultivation, thyme does not demand many requirements. It prefers dry, arid, sunny regions and sandy, calcareous soils; it is a rustic plant found in poor soils and does not like moist, compact soils (Castro & Chemale, 1995).

Considering that germination and initial growth are important to plants and influence their future, and that when these stages occur in an environment saturated with toxic metals there is a great chance of failure and death, the objective of this study was to evaluate the toxicity of cadmium on the seed germination and initial growth of thyme.

2. Material and methods

To evaluate the toxic effect of Cd on the germination process, the thyme seeds (*Thymus vulgaris* L.) were sown on a paper substrate moistened with aqueous solutions of cadmium chloride ($\text{CdCl}_2 \cdot 5\text{H}_2\text{O}$), at concentrations of zero (control), 15, 30, 45, and 60 mg L^{-1} . For the control (level zero), only distilled water was used. The toxic effect of Cd was evaluated using the tests listed below:

Germination: conducted based on four repetitions of 100 seeds distributed in plastic boxes, on germitest paper moistened with distilled water or cadmium sulfate solution (2.5 times the weight of the paper). After sowing the seeds, the plastic boxes were maintained in BOD chambers at a constant temperature of 20 °C and 8 h of light and 16 h of dark. Counts were made on days seven and 21 (when the test ended). It was evaluated the normal and abnormal seedlings and the results were expressed as percentages and the results were expressed as percentages (Brasil, 2009).

First count: conducted together with the germination test, where the percentage of normal seedlings was determined on day seven of the test.

Germination speed index (GSI): germinated seeds were counted daily at the same time. The criterion for germination was the protrusion of the radicle and the germination speed index was calculated based on the formula in Maguire (1962).

Seedling length: normal seedlings were obtained by sowing four repetitions of 20 seeds. Rolls of paper containing the seeds were kept in a germination chamber for seven days, at a temperature of 20 °C. Total length, shoot length and root length of 10 seedlings were randomly evaluated for each repetition using a millimeter ruler. The average length of the seedlings was obtained by adding the number of measurements of each repetition and dividing this by the number of normal seedlings measured, with the results expressed in centimeters (cm).

Dry mass of seedlings: first, the fresh weight was obtained from 10 previously measured seedlings, for four replicates, and then maintained in papers bags in a dryer at a temperature of 60 °C until reaching a constant weight (48 h). Subsequently, the seedlings were weighed on a precision balance (to 0.001 g) and the results were expressed in milligrams (mg).

Tolerance index (TI): was determined with the formula given by Iqbal and Rahmati (1992) (Ahmad et al., 2012).

$$\text{Tolerance index (T.I.)} = \frac{\text{Mean root length in metal solution} \times 100}{\text{Mean root length in control}}$$

Data analysis: the experimental design was completely randomized, where treatments consisted of different concentrations of the solutions. The variables expressed as percentages were converted to $\arcsin \sqrt{x/100}$. The data were submitted to an analysis of variance using the F test and, when significant, a regression analysis was performed using the program Sisvar (Ferreira, 2011).

3. Results and discussion

The analysis of variance indicated significant differences ($p < 0.05$) in function of the Cd concentrations for all variables except seedling dry mass (Table 1).

In the absence of Cd, an average of 88% normal seedlings and 7% abnormal seedlings were observed for the germination test (Figures 1 e 2). These values were lower for the 45 mg L⁻¹ concentration and reached 13% normal seedlings and 65% abnormal seedlings for the highest concentration used (60 mg L⁻¹).

The morphological analysis of the seedlings found that the presence of cadmium resulted in an increase in abnormal seedlings, revealing the toxic potential of this metal at the cellular level. Among the seedling abnormalities observed for the higher concentrations used, atrophy or the absence of a primary root were notable, as well as a thin, weak and contorted primary root (Figure 2b). According to Rossi and Lima (2001), the phytotoxic effect of cadmium promotes disorderly development and cellular differentiation, resulting in abnormal plants and decreasing the percentage of normal plants during germination.

Similar results were found by El Rasafi et al. (2016), who analyzed the tolerance of bean seeds to Cd (10 a 1000 mg L⁻¹). The authors found a significant reduction in the percentage of normal seedlings starting at 20 mg L⁻¹ of Cd. Similarly, Ahmad et al. (2012) found that this metal significantly affects the germination of wheat seeds and Gharebaghi et al. (2017) concluded that the germination of basal was inhibited in comparison to the control when using 20 mg L⁻¹ of Cd. In addition, Silva et al. (2017) evaluated the ecotoxicological effects of Cd on the germination of *Schinus terebinthifolius* found that low concentrations (0.2 mM) resulted in high inhibition percentages for germination and development of the aerial part and roots. According to Santos et al. (2013) and El Rasafi et al. (2016), the higher the amount of Cd in the soil the lower the germination rate, and inhibition of toxic metals depends on the concentration used, the metal itself, and the plant species.

The presence of toxic metals at the moment of germination can compromise the absorption of water, and inhibit the absorption of the solution, which can influence the development of the seedling (Liu et al., 2012). The inhibition of germination can occur due to a failure in mobilizing the endosperm, which is caused by a decline in α -amylase, acid phosphatase and alkaline phosphatase activity, and a small change in β -amylase activity, resulting in the failure of the mobilization of Cd (Kalai et al., 2014). In addition, the accumulation of Cd in plant cells can have effects at the biochemical and molecular levels by damaging biomolecules and causing oxidative stress, which effects plant development, such as inhibiting germination and growth (Hossain et al., 2011).

For the first count germination test, there was a decrease in the percentage of normal seedlings (81% to 11%) as the Cd concentration increased (Figure 3a). However, the germination speed decreased only when the concentration of Cd in the substrate was 60 mg L⁻¹ (Figure 3b).

Similar to this study, Ahmad et al. (2012) observed a significant reduction in the germination speed of wheat seeds starting at 50 mg L⁻¹ of Cd. In addition, Pires et al. (2016) and Borges et al. (2016) found that Cd, at whatever concentration tested, negatively affected the performance of sesame and *Brachiaria decumbens* seeds, respectively, by reducing the germination speed.

Delayed germination can occur due to the protective role the seed integument plays, which can block and retain toxic metals on its surface (Sun & Luo, 2014). A reduction in germination speed can be related to a decrease in enzymatic activity linked to embryo growth and the protrusion of the radicle, since Cd disrupts development and cellular differentiation and growth by changing the activity of the enzyme peroxidase (Santos et al., 2013).

For total length of the aerial part and the roots of thyme, there was a reduction in size with an increase in the metal concentration (Figure 3a). There was a reduction in total length of the seedlings from 3.78 cm (control) to 2.22 cm (60 mg L⁻¹) (Figure 4a). Similarly, the length of the aerial part decreased from 1.26 cm (control) to 0.57 cm and the length of the roots

decreased from 2.52 (control) cm to 1.64 cm for the highest concentration of Cd used (60 mg L⁻¹). Further, there was no significant difference in the dry mass of the seedlings (Figure 4b).

The results of the present study corroborate those of Gharebaghi et al. (2017), who analyzed the response of basal to Cd (0 a 20 mg L⁻¹) and found that the number of leaves, plant height, and root length were clearly smaller when the metal concentration increased. Similarly, Saderi and Zarinkamar (2012) observed a reduction in growth of *Matricaria chamomilla* seedlings, mainly the roots, exposed to high concentrations of Cd (0 to 180 µM). Further, Grigoraş and Stratu (2015) observed a significant unfavorable influence ($p < 0.05$) on seedling root length (at a concentration of 10 mg L⁻¹) and plant length of *Dianthus chinensis* at a concentration of 10 mg L⁻¹. Cadmium also significantly interfered in the growth of the aerial part and roots of *Schinus terebinthifolius* starting at a concentration of 0.2 mM (Silva et al., 2017).

The presence of toxic metals can cause changes in size, shape and arrangement of cortical parenchyma. These changes indicate that this metal interferes with the maturation rate of the root, which is possibly because these contaminants disturb the hormonal equilibrium of the plant. The cell walls that thicken the root can provide more area to retain toxic metals, reducing their translocation to the aerial part (Gomes et al., 2011). Seedling growth is affected because the metals disrupt the metabolism of the plants due to interactions with enzymes and biochemical reactions that occur in the plant (Ashraf et al., 2011).

The tolerance index to 15, 30, 45 and 60 mg L⁻¹ was 90, 69, 70 and 63% (Figure 5), indicating that the increase in Cd concentration in the substrate caused a decrease in the root length. This index reflects the ability of the seedlings to grow in environments with high concentrations of the metal and, consequently, it is recommended that thyme should only be cultivated in areas with low Cd levels.

Many studies report that Cd is toxic to most plants, even at low concentrations. The roots are the first part of the seedling to come in contact with contamination and are more sensitive to metal toxicity compared to the aerial part (Yang et al., 2010). This element is absorbed by the roots, causing phytotoxicity by influencing cell growth in the zone of elongation in the root and impeding absorption mechanisms and the conduction of water and nutrients (Bian et al., 2013).

Finally, toxic metals adversely interfere with the growth, distribution and biological cycle of plant species, which makes it necessary to search for plants that have tolerance mechanisms to use in contaminated areas as an alternative for environmental remediation. The same plant may exhibit different behaviors in a contaminated soil, and the response to contamination can vary based on the characteristics of the species, the stress, and the conditions of the specific area. Thus, more detailed studies are needed to establish the maximum amount of Cd that thyme seeds can tolerate for germination and growth in environments contaminated with this toxic metal.

4. Conclusion

The presence and accumulation of Cd in the substrate of the culture played an inhibitory role in the seed germination and initial seedling growth of thyme, and the intensity of inhibition was directly proportional to the concentration of the solutions employed.

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Table 1. Summary of the analysis of variance for the variables normal seedling (NS), abnormal seedling (AS), first count (FC), germination speed index (GSI), total length (TL), shoot length (SL), root length (RL), dry mass (DM), and indices (TI) of thyme seedlings (*Thymus vulgaris*) exposed to different concentrations of cadmium.

Source of variation	Degrees of freedom	Mean square								
		NS	AS	FC	GSI	TL	SL	RL	DM	TI
Treatment	4	4268.6*	2515.2*	3595.7*	46.13*	1.72*	0.33*	0.60*	0.013	921.7*
Residue	15	15.61	14.61	15.40	6.26	0.10	0.01	0.06	0.008	90.49
CV (%)	-	5.53	18.34	6.14	5.83	8.49	15.21	12.99	18.63	12.04

* Significant at 5% probability by the F test. CV = Coefficient of variation

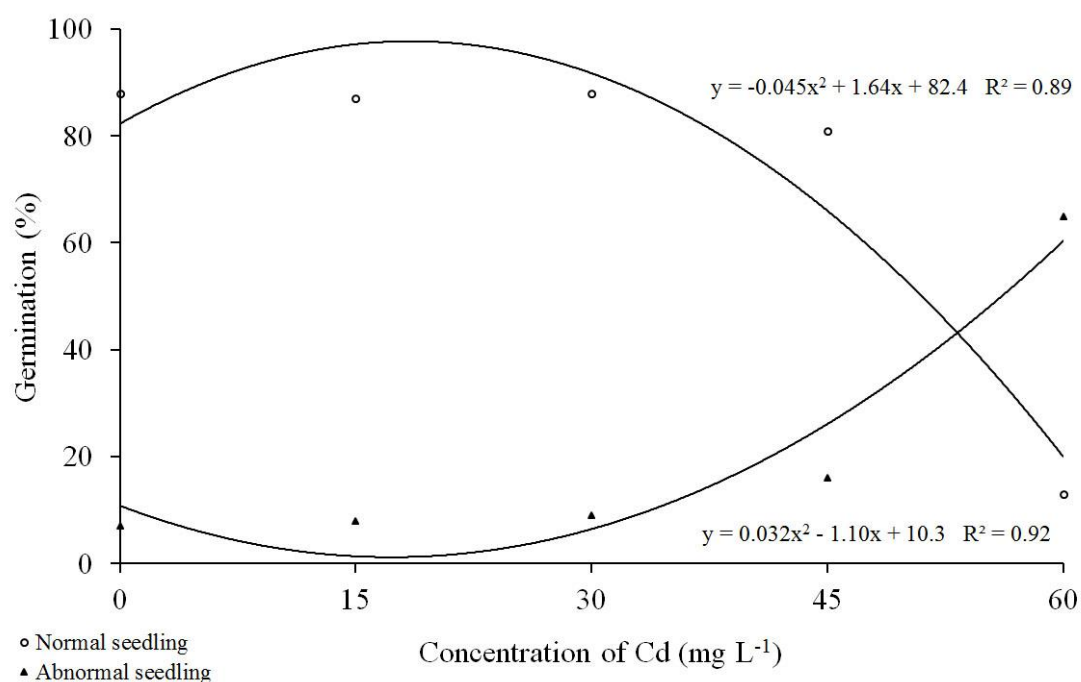


Figure 1. Germination of thyme seeds (*Thymus vulgaris*) exposed to different concentrations of cadmium.

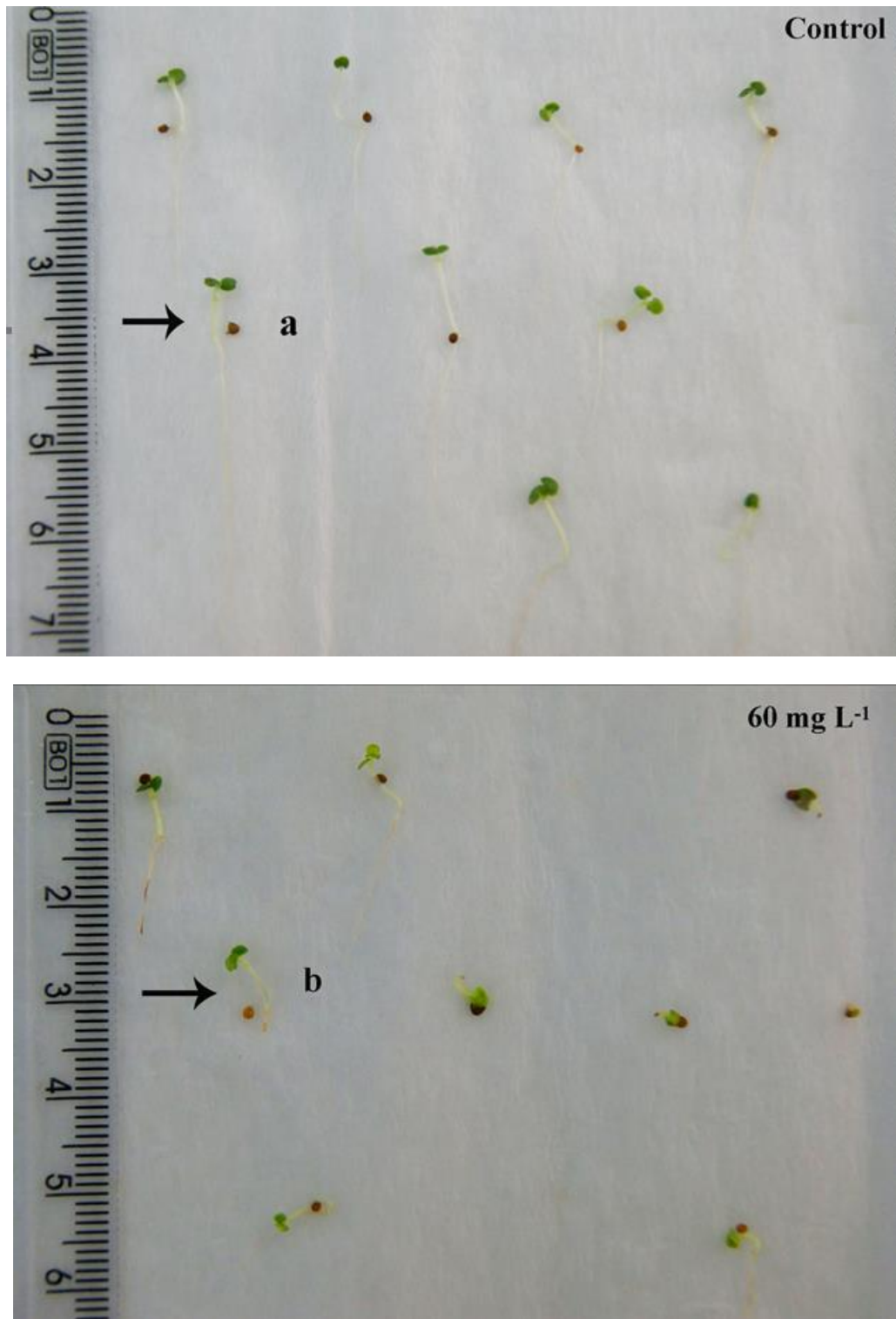


Figure 2. Normal seedling of thyme obtained from seeds germinated in water on day seven (a); abnormal seedling of thyme (*Thymus vulgaris*) obtained from seeds germinated in a solution of 60 mg L⁻¹ of cadmium on day seven (b).

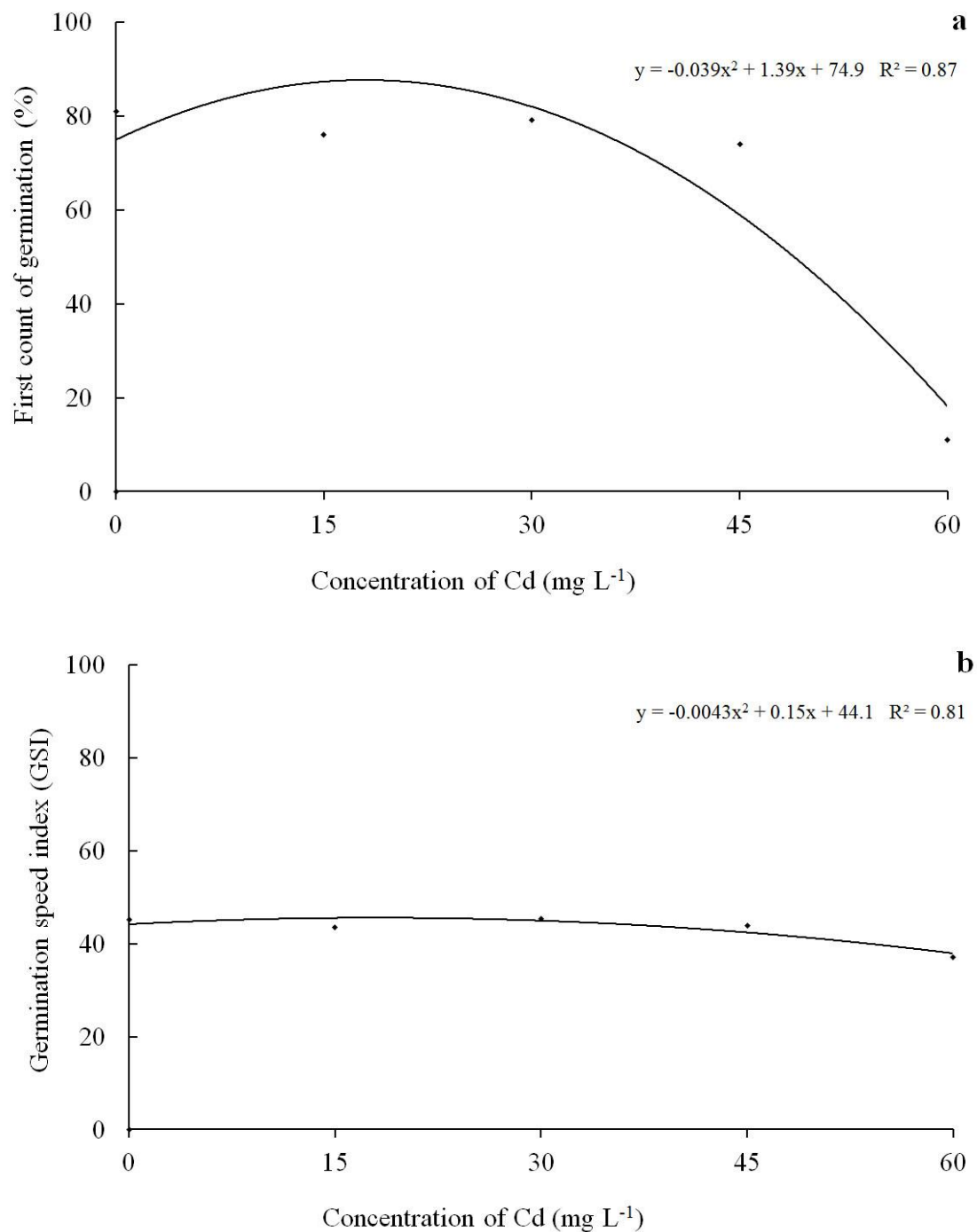


Figure 3. First count (a) and germination speed index (b) of thyme seeds (*Thymus vulgaris*) exposed to different concentrations of cadmium.

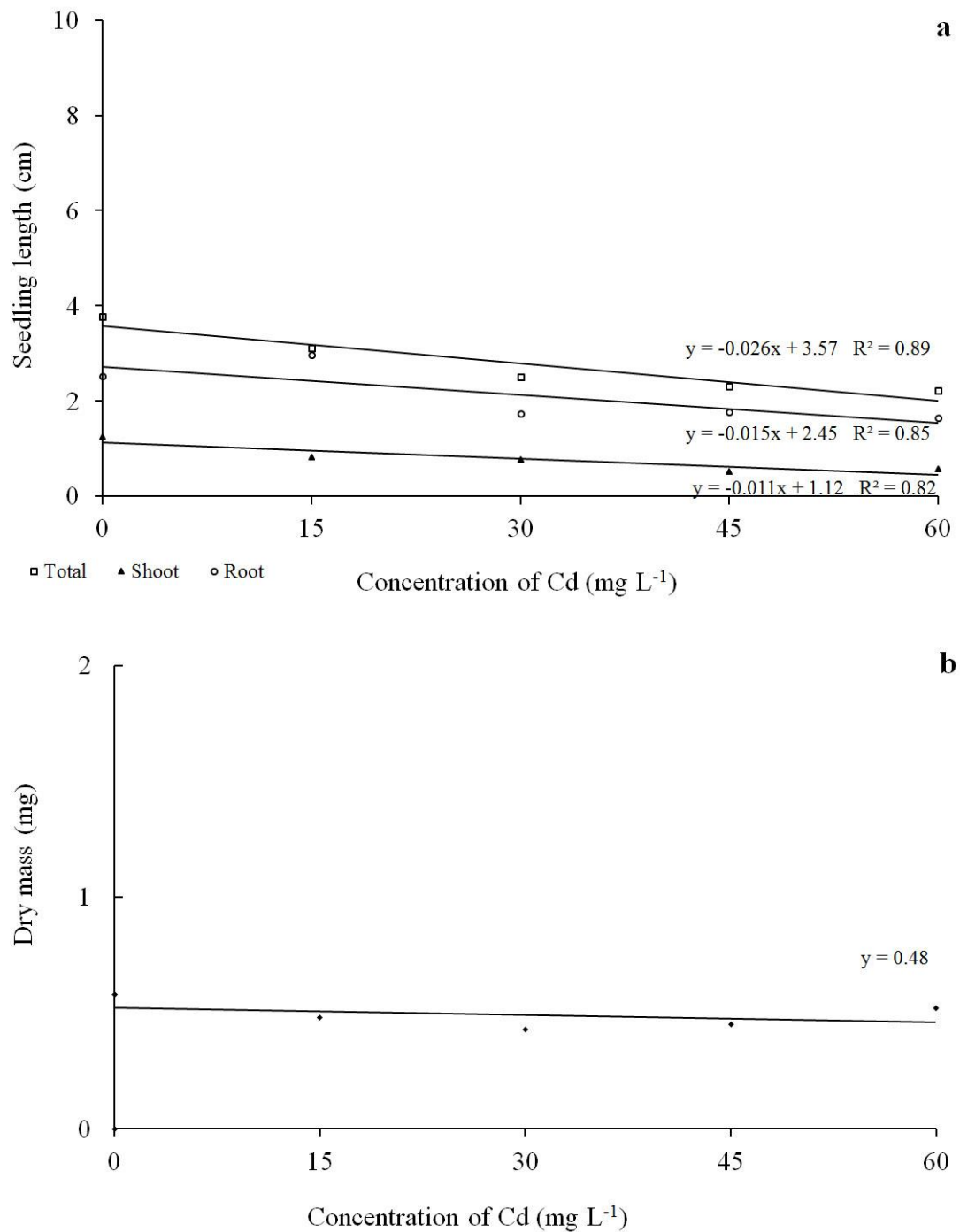


Figure 4. Length (a) and dry mass (b) of thyme seedlings (*Thymus vulgaris*) exposed to different concentrations of cadmium.

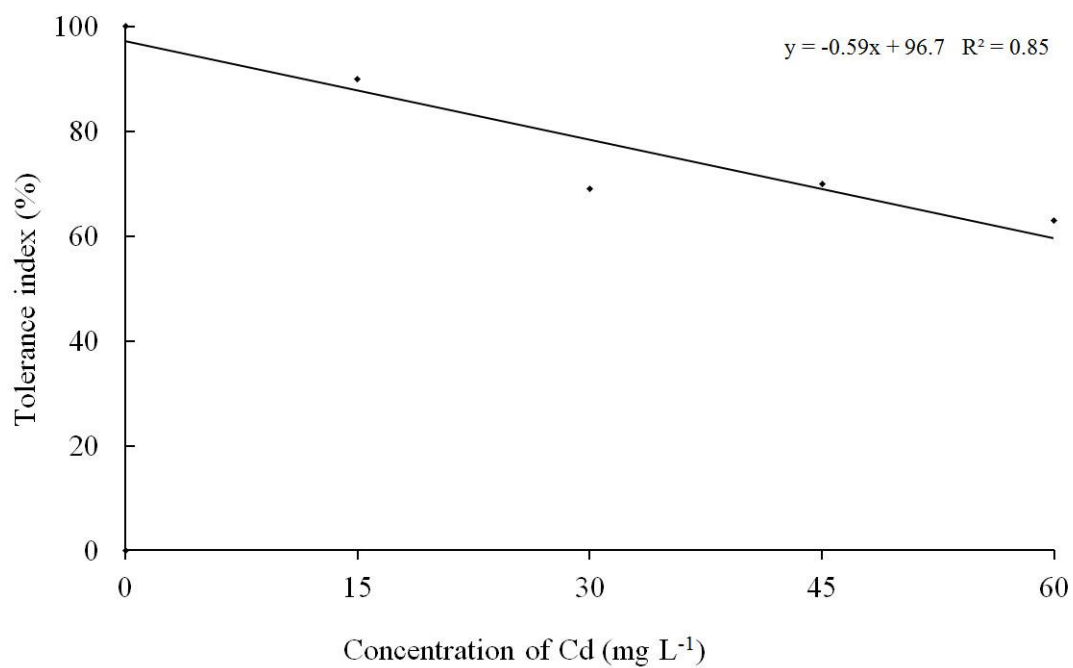


Figure 5. Tolerance index of roots of thyme seedlings (*Thymus vulgaris*) exposed to different concentrations of cadmium.